Extrahypothalamic Pathways of a Neurosecretory System: Their Role in Neurotransmission

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Antidiuretic and oxytocinergic principles, which stem from the paraventricular nucleus (PVN) and the supraoptic nucleus (SON), are transported via the hypothalamo-neurohypophysial tract to the neural lobe, and are subsequently released in the blood. This was the major conclusion of Bargmann and Scharrer (2) in their paper on “The site of origin of the hormones of the posterior pituitary.” With this paper and an earlier one by Bargmann (1) the concept of neurosecretion (19) was eventually accepted. Only a few years later, Barry (3) suggested on morphological grounds that Gomori-positive fibers would terminate in the brain by means of “synapses neurosécrétoires,” which he emphasized were likely to have a physiological significance.

With immunocytochemistry for the specific localization and detection of the hormones involved, it became possible to corroborate and extend these excellent studies. Therefore, in the present paper evidence will be presented that arginine-vasopressin (AVP) and oxytocin (OXT), two neurohypophysial hormones, are widely distributed in the central nervous system (CNS), and function as neurotransmitters in many brain areas.
regions. The putative physiological function of these peptidergic neurotransmitters will be discussed.

I. INNERVATION OF THE CNS BY VASOPRESSIN AND OXYTOCIN FIBERS

Apart from the classical hypothalamo-neurohypophysial system consisting of the PVN and SON and their AVP- and OXT-containing pathways towards the neural lobe, specific immunocytochemical techniques revealed another nucleus that contained vasopressin only, the suprachiasmatic nucleus (SCN) (23, 27). Meanwhile, the increase in sensitivity of the immunocytochemical techniques employed and optimization of the fixation procedures made possible the visualization of hitherto unknown AVP and OXT pathways in the CNS (5, 22).

The innervation of the brain by AVP and OXT fibers is largely limited to phylogenetically older brain regions, such as the spinal cord and brain stem (in the rat brain, predominantly OXT) and, except for primates, the limbic brain regions, such as the hippocampus and septum (mostly AVP) (see Figs. 1 and 2). In addition, each circumventricular organ, or its immediate surroundings, seems to receive

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Fig. 1. The major oxytocinergic pathways largely arising from the PVN (see text). A, amygdala; DBB, diagonal band of Broca; DMH, dorsomedial nucleus of the hypothalamus; DVC, dorsal vagus complex; LC, locus coeruleus; LH, lateral habenula; LS, lateral septum; MB, mammillary body; ME, median eminence; MFB, medial forebrain bundle; MS, medial septum; NA, nucleus ambiguus; OB, olfactory bundle; OVL, organum vasculosum of the lamina terminalis; P, pineal; PNB, parabrachial nucleus; PVG, periventricular grey; PVN, periventricular nucleus; RD, dorsal raphe nucleus; SC, superior colliculus; SFO, subfornical organ; SN, substantia nigra; SON, supraoptic nucleus; TT, taenia tectae; VHi, ventral hippocampus; VTA, ventral tegmental area.
Fig. 2. The major vasopressinergic pathways in the brain with their most likely origin (see text). The pathways of the PVN are indicated by (----); pathways of the SCN (▲) are indicated by (-----). The vasopressinergic cell groups found only after colchicine treatment are indicated by large black dots, while the pathways of the BST are indicated by (-----). The question mark indicates that the source of the vasopressin innervation in this area is still unknown. See the legend of Fig. 1 for abbreviations.

an AVP or OXT input. The large number of different brain regions innervated by AVP and OXT required careful analysis in order to determine the nuclei from which these fibers were derived. Retrograde tracing in combination with immunocytochemistry made it possible (24) to demonstrate that the dorsal vagal complex largely receives its AVP and OXT input from the parvocellular part of the PVN. In search of the origin of the vasopressinergic innervation of the lateral septum (LS), a lesion in the SCN (12) and PVN (8) showed that neither of these nuclei projected to the LS. It resulted, however, in the revealing of the vasopressinergic projections of the SCN towards the organum vasculosum of the lamina terminalis, the nucleus paraventricularis of the thalamus, and the dorsomedial hypothalamic nucleus (12) (Fig. 2). In addition, it also turned out that the majority of the AVP and OXT innervation in the brain stem disappeared after PVN lesioning. Moreover, also in most of the other brain regions the OXT innervation disappeared, which indicates that the PVN is the major source of the OXT innervation of the brain, while only the caudal AVP projections were probably derived from the PVN (8). Following colchicine injection, AVP-containing cells were found, e.g., in the bed nucleus of the stria terminalis (BST) (28), in exactly the same position as retrogradely HRP-filled cells after LS injection (8). Therefore, the BST was lesioned and subsequent AVP immunocytochemistry revealed a strong diminution of AVP innervation
of the LS. Consequently the BST was proposed to be the most likely source of AVP present in the LS (Fig. 2).

The reason why attention was focussed on the LS is that, apart from being the most densely AVP-innervated part of the brain, it is also innervated in a sexually dimorphic fashion. Male rats have a denser AVP plexus in the LS than their female counterparts (9). The density of the AVP innervation turned out to be dependent on the presence of testosterone during development. In addition, in adulthood it was observed that after castration of adult male or female rats AVP innervation had disappeared altogether after a 15-week period, e.g., from the LS, the lateral habenular nucleus and the amygdala (10).

II. VASOPRESSIN AND OXYTOCIN AS NEUROTRANSMITTERS

The dense AVP and OXT innervation of several brain regions

Fig. 3. Vasopressin-containing terminal forming a synapse (long arrow) with unlabeled dendrite in the nucleus of the solitary tract. Note the ±90 nm dense core vesicles (short arrows). Bar = 500 nm.
where these fibers were found to form many perineuronal structures and extensive ramifications was—as Barry (3) had already suggested—indicative of the presence of synaptic terminals. Indeed, immunoelectronmicroscopy of AVP and OXT in various brain regions (6, 30) demonstrated the presence of these neurohypophysial peptides, also in synaptic structures in the brain (Fig. 3).

In order to prove that these peptides could be released like neurotransmitters, an in vitro incubation procedure was developed, which demonstrated (7) potassium-stimulated release of AVP and OXT from the region of the septum and nucleus of the solitary tract and that this release is [Ca$^{2+}$]-dependent, just like the classical amine-ergic transmitters. Moreover, it was demonstrated that release only occurred in regions where these fibers exhibited synaptic specializations. No potassium-stimulated release of OXT or AVP could be observed from the region between the PVN and SON, where many AVP and OXT fibers are present with varicosities, but not exhibiting synaptic specializations. Thus, the actual synaptic release of AVP and OXT was demonstrated in vitro. In addition, since several reports now describe electrophysiological actions of AVP and OXT in the regions where these fibers terminate (11, 13, 16), there is little doubt that these peptides are able to function as neurotransmitters in the CNS.

III. POSSIBLE FUNCTION OF VASOPRESSIN AND OXYTOCIN IN THE CNS

The central effects of AVP and OXT on passive avoidance behavior and monoamine metabolism (15, 29), blood pressure (cf. 4), and on thermoregulation (14) are well documented. Apart from these effects of centrally injected peptides, little is known about the function of AVP and OXT in the CNS.

Since the anatomy of the system is now fairly well known, we shall try to draw some conclusions. Exclusively on the basis of the fact that AVP originates from several nuclei, and reaches many more brain regions than OXT, it could be asserted that AVP is likely to be involved in the regulation of many more functions than OXT. Moreover, much of the AVP innervation of the brain appears to be sexually dimorphic,
which suggests that AVP may affect sex-linked processes. It is suggested, for instance, that AVP might be involved in the regulation of mounting behavior (2J). Since the AVP/OXT fibers in caudal brain parts predomi-

Fig. 4. Two alternating 50 μm vibratome sections in the lower brain stem. A: stained for OXT with extensive innervation in the A1 and A2 areas (arrows). B: stained for noradrenaline demonstrating noradrenergic cell bodies in the A1 and A2 regions. Note the overlap between OXT innervation and the presence of noradrenaline cell bodies.
nantly innervate brain structures involved in the regulation of autonomic processes, and since the OXT fibers are far more numerous in caudal brain regions, it seems logical to assume that OXT plays a role in the regulation of such autonomic processes. Since most of the caudal OXT/AVP innervation is derived from the PVN, this nucleus is bound to be crucial in controlling these autonomic functions (25). Anatomical tracing studies have suggested a relationship between some brain regions that receive an AVP or OXT input, and the PVN and/or SON. It has been demonstrated for the A1 region, for instance, that cells from this region project towards the PVN and SON (18). The dense OXT innervation in this noradrenergic nucleus (Fig. 4) makes it likely that these cells, projecting to the SON and PVN, receive AVP or OXT input.

In addition, injection of HRP into the PVN resulted in retrograde labelling of cells in the LS and amygdala (20, 26). Recent evidence seems to indicate that LS cells project to AVP neurons in the PVN (17). Indeed, cell bodies in the LS that are retrogradely labelled after HRP injection in the PVN are innervated by AVP fibers (Croese and Buijs, unpublished result). These anatomical studies suggest that AVP and OXT in some parts of the CNS might be able to indirectly influence the release of AVP and OXT from the hypothalamo-neurohypophysial system into the general circulation.

SUMMARY

AVP and OXT have been demonstrated in many brain regions. AVP innervation seems to be more abundant in forebrain (limbic) regions, while OXT fibers more extensively innervate caudal brain regions. Evidence is presented that these peptides may serve as neurotransmitters in several brain regions. In addition, it is suggested that AVP and OXT in the CNS may be involved in the regulation of their own release into the general circulation.

REFERENCES